

Supplementary Material

General anesthetic conditions induce network synchrony and disrupt sensory boundaries in the cortex

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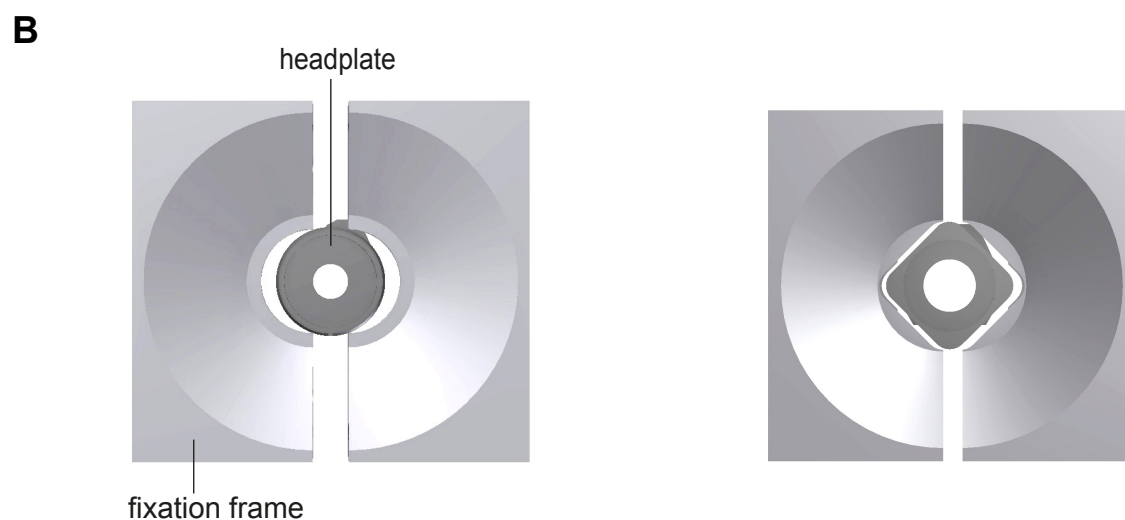
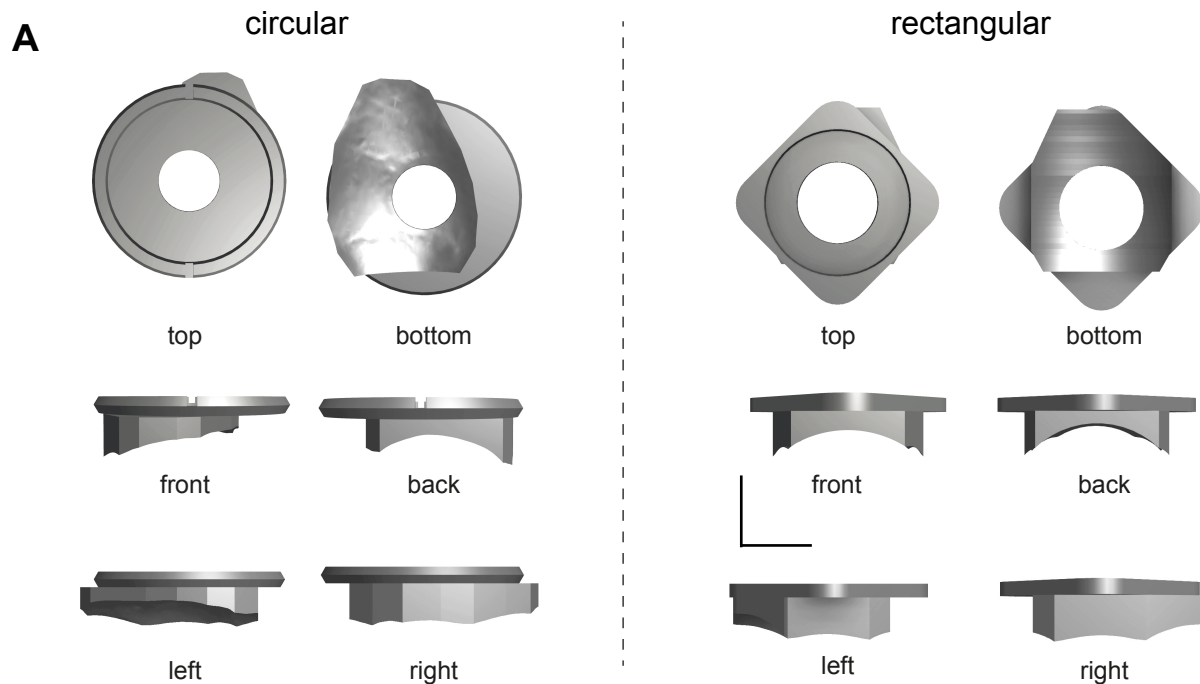
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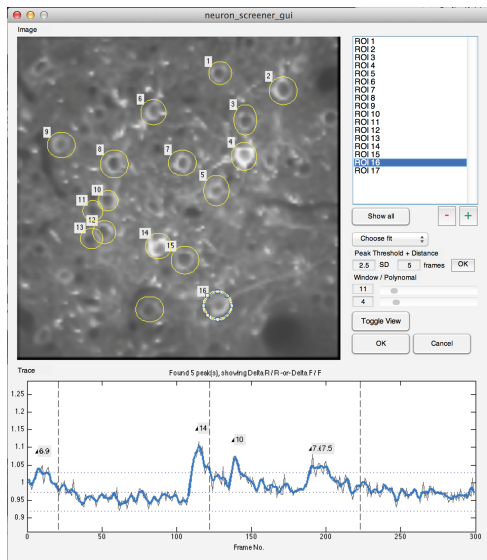
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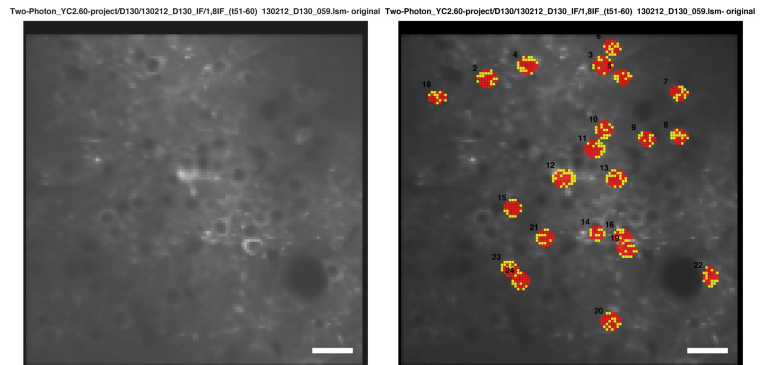


Supplementary Figure 1 Titanium headplates for chronic *in vivo* calcium imaging in the barrel cortex. (a) Two versions of titanium headplates for reproducible animal fixation under the microscope are shown from different angles. The circular design offers rotational flexibility around the vertical axis in case the animal needs to be rotated relative to the microscope stage. The two indents allow for orientation. The rectangular design allows maximum reproducibility of animal fixation and facilitates the revisiting of defined brain regions for chronic imaging. Scale bar: 5 mm (both axes). (b) Both headplate designs are shown in their corresponding fixation frames.

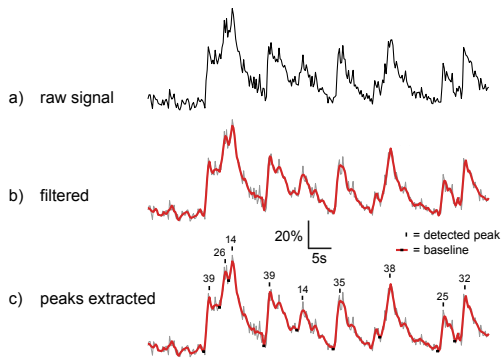
1. manual ROI selection



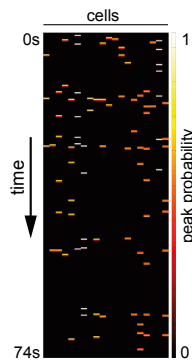
2. alignment (intra and inter stack) + ROI enhancement



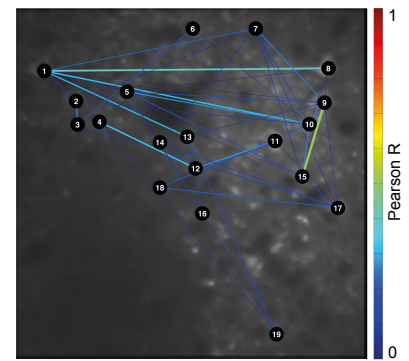
3. noise filtering and peak extraction



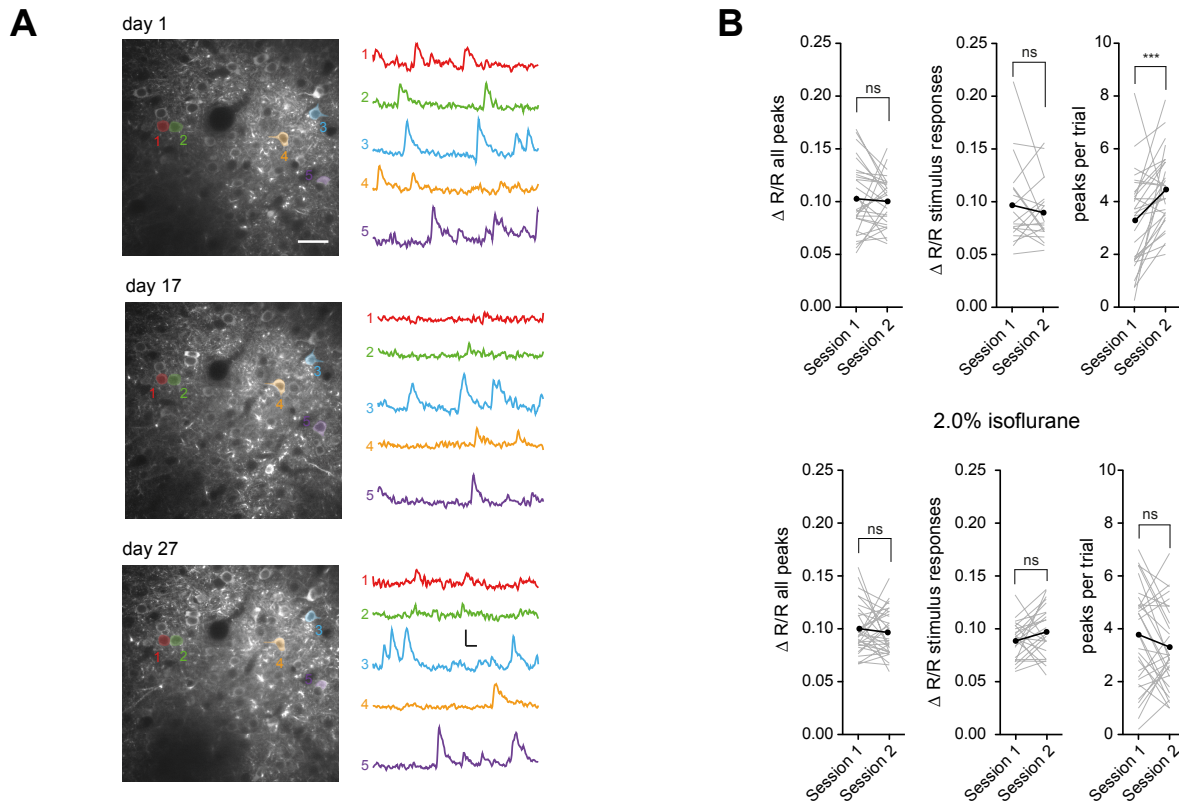
4. Peak probability calculation



5. Correlation analysis



Supplementary Figure 2 Workflow for image analysis. For details on calculations, we refer to the methods section. First, ROIs are drawn manually and parameters for subsequent signal analysis are chosen in a custom graphical user interface (1). Image stacks were then automatically corrected for inter- and intratrial frameshifts (not shown). To enhance signal-to-noise ratio and to reduce neuropil signal contamination, ROIs were automatically refined by pixel fluorescence correlation analysis (2). The extracted raw signal was then noise-filtered and peaks were automatically extracted with individual baseline correction for each peak (3). To eliminate detection bias of raster scanning, each peak was assigned a probability value according to its position in the field of view (4). Peaks were then analyzed for pairwise correlation (5) and stimulus responsiveness (not shown).



Supplementary Figure 3 Chronic in vivo calcium imaging (a) Chronic in vivo Ca^{2+} imaging in layer 2/3 of the barrel cortex. Images depict brain region for functional imaging on days 1, 17 and 27 (depth = $\sim 120 \mu\text{m}$ from the dura mater). One example trace per cell and day is shown. All neurons showed regular activity on all experimental days. All activity shown was recorded under 0.5% isoflurane anesthesia. Scale bar: $30 \mu\text{m}$. (b) Comparison of cells that were identified in two sessions during 0.5% and 2.0% Isoflurane anesthesia. Grey lines indicate single cells, black thick lines indicate group mean. Cells were compared for: $\Delta R/R$ for all peaks (including spontaneous and stimulus evoked events), $\Delta R/R$ of stimulus responses (stimulus evoked events only) and number of events per trial (peaks per trial). Activity parameters stayed mostly stable in between imaging sessions (27.8 ± 2 cells (Mean \pm SEM), 4 mice, Wilcoxon matched-pairs signed rank test, $p > 0.05$; $p = 0.0003$ for peak per trial Session 1 vs. Session 2 at 0.5% Isoflurane).

Supplementary Table 1

Anesthesia states

- = absent; +/- = weakly present or absent; + = moderate; ++ = strong

Isoflurane

Anesthetic depth	erect posture	spont. limb movements	spont. whisker movements	reaction to paw and tail pinch	breathing
awake	++	++	++	++	physiologic
light (sedation or recovery)	++	+	++	++	physiologic
medium	-	-	-	+/-	~ 2 Hz
deep	-	-	-	-	~ 0.5 Hz

Ketamine and Ketamine/Xylazine

	erect posture	spont. limb movements	spont. whisker movements	reaction to paw and tail pinch
light (recovery)	+	+	++	++
medium	-	-	+	+/-
deep	-	-	-	-

Urethane

Under urethane anesthesia, all animals showed no reaction to paw pinch and no spontaneous whisker or limb movements. After the first two injections, the breathing frequency remained at the physiological level. After the third injection, the breathing frequency started to decrease continuously.

Supplementary Table 2

Isoflurane correlation analysis				whisker	mouse n
	Low	Medium	High	Recovery	
n independent	22	12	12	10	7
Minimum	5	9	5	4	
Maximum	34	20	20	22	
Median	10	11	11	9	
Mean	11.23	11.83	11.58	10.6	
Std Dev	5.887	3.433	4.963	5.719	
Isoflurane correlation analysis				sound only	
	Low	Medium	High	Recovery	
n independent	3	3	3	3	3
Minimum	7	8	9	4	
Maximum	8	20	14	9	
Median	7	11	10	8	
Mean	7.333	13	11	7	
Std Dev	0.5774	6.245	2.646	2.646	
Ketamine + Xylazine					
	K+X				
n independent	12				8
Minimum	14				
Maximum	52				
Median	25.5				
Mean	27.75				
Std Dev	12.29				
Ketamine only					
n independent	3				3
Minimum	22				
Maximum	44				
Median	22				
Mean	29.33				
Std Dev	12.7				
Urethane					
	1	>1			
n	3	3			3
Minimum	11	15			
Maximum	25	25			
Median	15	22			
Mean	17	20.67			
Std Dev	7.211	5.132			